SCIENTIFIC ABSTRACT

U937, a CD4+ cell line derived from human promonocytic cells, was made resistant to HIV-1 by the introduction of a DNA construct that contained 3 independent antisense sequences directed against 2 functional regions, TAR and *tat/rev*, of HIV-1. Stable transfected cells expressed all three antisense transcripts, and these transcripts accumulated in the nucleus. These cells were subjected to 2 successive challenges with HIV-1 (BAL strain). The surviving cells showed normal growth characteristics and have retained their CD4+ phenotype. *In situ* hybridization assays showed that essentially all of the surviving cells produced high levels of antisense RNA. When the surviving cells were challenged with HIV-1 (BAL strain) no detectable p24 antigen was observed, and PCR-amplified HIV-1 gag nucleic acid sequences were not detected indicating a profound inhibition of HIV-1 replication. As a further demonstration that the antisense RNA directed against HIV-1 was present and functioning in these transfected immune cells, tat-activated expression of the gene coding for chloramphenicol transacetylase was shown to be specifically inhibited in cells expressing either of the tat-antisense sequences or the tar-antisense sequence singly. Also, the cells containing these three genetic antisense genes did not support the replication of three independently isolated HIV-1 strains.

These antisense sequences have been embedded into cloned human U1 RNA genes. These three U1/HIV-1 antisense genes have been combined into a triple U1/HIV-1 antisense cassette and incorporated into a Moloney Murine Leukemia Virus (MMLV)-based transducing vector.

In this protocol we propose to isolate from circulation a population of cells enriched for the CD34+ antigen (peripheral blood stem cells or PBSC). These cells will be isolated from HIV-1 infected subjects previously treated with granulocyte colony stimulating factor (GCSF). They will be transduced with Enzo's triple U1/HIV-1 antisense MMLV vector. The transduced cells containing the anti-HIV-1 antisense genes will then be infused into the HIV-1 subject from which they were originally derived. The end points of this study are: the safety of the procedure; the extent of engraftment and proliferation of this engineered cell population; and the relative efficacy of two separate dosing protocols. Our study will employ up to 8 patients.